PATENT
PC6947AJTL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE:	U.S. PATENT NO.: 5,089,480	:
ISSUED:	FEBRUARY 18, 1992	:
TO:	STEPHEN P. GIBSON ET AL.	:
FOR:	ANTIPARASITIC AGENTS	:
FROM:	SERIAL NO. 142,888	:
OF:	JANUARY 11, 1988	:

#22

Hon. Commissioner of Patents and Trademarks
Box Patent Extension
Washington, DC 20231

Sir:

APPLICATION FOR EXTENSION OF
PATENT TERM UNDER 35 U.S.C. §156

Transmitted herewith is the application of PFIZER INC., dated September 25, 1996, for extension of the term of United States Patent No. 5,089,480 under 35 U.S.C. §156, together with a duplicate of the papers thereof, certified as such.

Please charge the sum of $1,060.00 to Deposit Account No. 16-1445. Please also charge any additional fees which may be required by the filing of this application for extension of patent term, or credit any overpayment, to Deposit Account No. 16-1445. Two copies of this paper are enclosed.

Respectfully submitted,
PFIZER INC.

Date: September 25, 1996

By: J. Trevor Lumb

J. Trevor Lumb, Its Agent
Reg. No. 28,567
Tel.: (212) 573-2521

Pfizer Inc.
Patent Department
235 East 42nd Street
New York, NY 10017-5755

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Sir:

CERTIFICATION

I hereby certify that attached hereto is a duplicate copy of the application papers of PFIZER INC., dated September 25, 1996, for extension of the term of United States Patent No. 5,089,480 under 35 U.S.C. §156.

Respectfully submitted,

Date: September 25, 1996

J. Trevor Lumb

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Hon. Commissioner of Patents and Trademarks
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Washington, DC 20231

Sir:

APPLICATION FOR EXTENSION OF THE TERM OF UNITED STATES PATENT NO. 5,089,480 UNDER 35 U.S.C. §156

Your applicant, PFIZER INC., a corporation organized and existing under the laws of the State of Delaware, and having a place of business at 235 East 42nd Street, New York, NY 10017, U.S.A., represents that it is the owner of the entire right, title and interest in and to Letters Patent of the United States No. 5,089,480, granted to STEPHEN P. GIBSON, ALEXANDER C. GOUDIE, KELVIN S. HOLDOM and JOHN D. BU'LOCK on the 18th day of February 1992, for ANTIPARASITIC AGENTS, by virtue of an assignment, recorded in the United States Patent and Trademark Office on the 11th day of January, 1988, at Reel 4876, Frame 0490.

Pursuant to the provisions of 37 C.F.R. §1.730, your applicant hereby applies for an extension of the term of said United States patent under 35 U.S.C. §156 of 527 days, based on the materials set forth herein and in the accompanying papers. In the materials which follow herein, paragraph numbers correspond to the paragraph numbers in 37 C.F.R. §1.740(a).

(1) The approved product is DECTOMAX, which is further identified as follows.

Chemical Name

25-des-sec-butyl-25-cyclohexyl-avermectin B1a

Generic Name

Doramectin

Pfizer Inc. Code Number

UK-67,994

Molecular Formula

$C_{50}H_{74}O_{14}$

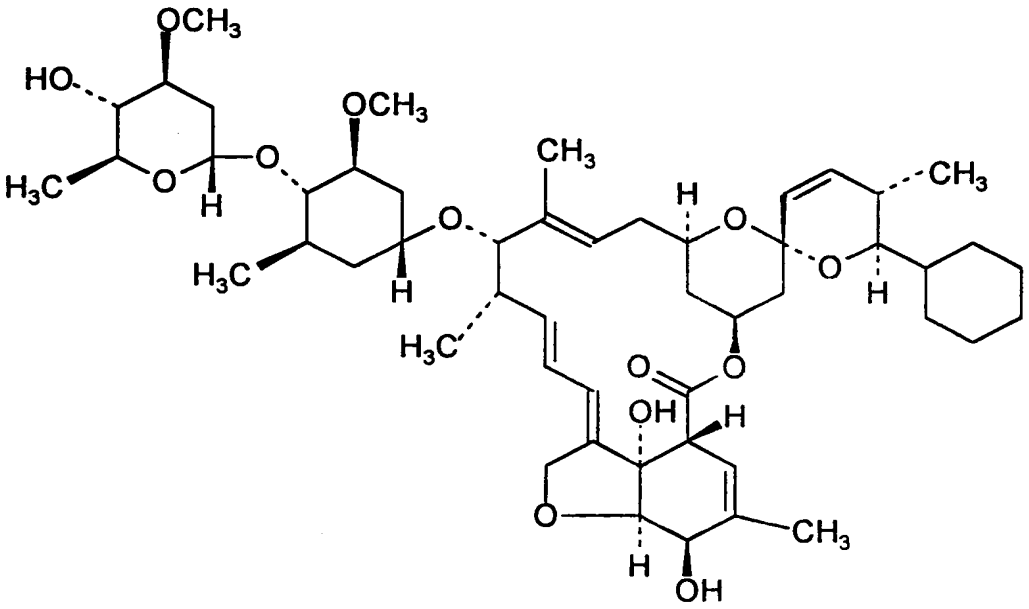
Molecular Weight

899.1

Physical Description

White solid; m.p. 165-167°C; soluble in common organic solvents but sparingly soluble in water.

Chemical Formula



(2) DECTOMAX was subject to regulatory review under section 512 of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. 360(b).

(3) DECTOMAX received permission for commercial marketing or use under section 512 of the Federal Food, Drug and Cosmetic Act, 21 U.S.C. 360(b), on July 30, 1996.

(4) The active ingredient in DECTOMAX is 25-des-sec-butyl-25-cyclohexyl-avermectin B1a (doramectin). Said active ingredient has not been previously approved for commercial marketing or use under the Federal Food, Drug and Cosmetic Act, the Public Health Service Act or the Virus-Serum-Toxin Act.

(5) This application is being submitted within the sixty day period permitted for its submission pursuant to 37 C.F.R. §1.720(f). The last day on which this application could be submitted is September 30, 1996. (The sixtieth day following permission for commercial marketing or use falls on September 28, 1996, which is a Saturday.)

(6) The patent for which an extension is being sought is identified as follows.

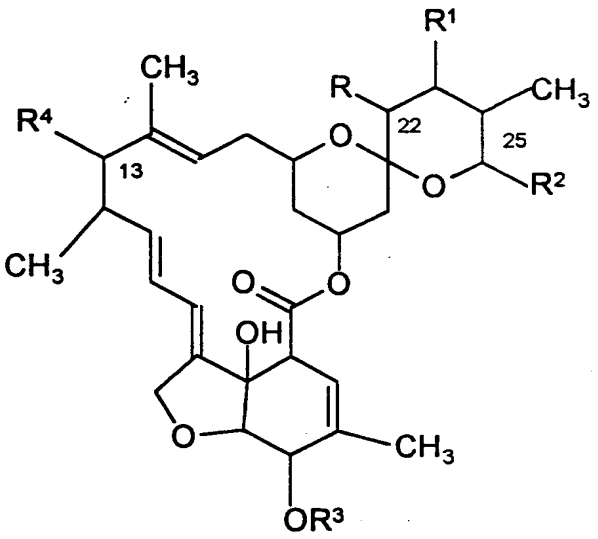
Inventors:	STEPHEN P. GIBSON, ALEXANDER C. GOUDIE, KELVIN S. HOLDOM and JOHN D. BU'LOCK
Patent No.:	5,089,480
Title:	ANTIPARASITIC AGENTS
Issued:	February 18, 1992
Expires:	February 18, 2009

(7) A copy of United States Patent No. 5,089,480, the patent for which an extension is being sought, is attached hereto as EXHIBIT A.

(8) No disclaimer or reexamination certificate has issued in United States Patent No. 5,089,480. A copy of the certificate of correction which issued in United States Patent No. 5,089,480 is attached hereto as EXHIBIT B, and a copy of the receipt of maintenance fee payment which issued in United States Patent No. 5,089,480 is attached hereto as EXHIBIT C.

(9) United States Patent No. 5,089,480 claims the approved product. Claims 1, 2, 17, 18 and 19 claim the approved product per se. Claims 33 and 34 claim compositions which contain the approved product, and which are of use for the approved use of the approved product. The manner in which each applicable patent claim reads on the approved product is as follows.

Claim 1 of U.S. 5,089,480 claims a genus of chemical compounds of the following chemical formula:

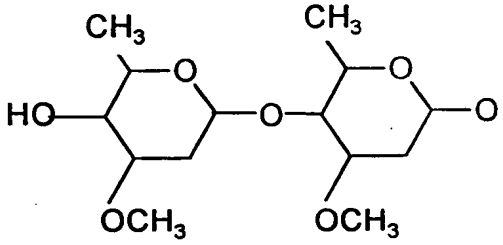


wherein R when taken individually is H; R^1 when taken individually is H or OH; R and R^1 when taken together represent a double bond;

R^2 is an alpha-branched C_4-C_8 alkynyl, C_3-C_8 alkoxyalkyl or C_3-C_8 alkylthio group; a C_5-C_8 cycloalkylalkyl group wherein the alkyl group is an alpha-branched C_2-C_5 alkyl group; a C_3-C_8 cycloalkyl or C_5-C_8 cycloalkenyl group, either of which may be substituted by methylene or one or more C_1-C_4 alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may be substituted by one or more C_1-C_4 alkyl groups or halo atoms;

R^3 is hydrogen or methyl;

R^4 is H or a 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy group of the formula:



In claim 1, when R and R^1 are taken together and they represent a double bond, R^2 is cyclohexyl, R^3 is hydrogen and R^4 is the 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy group, the claimed

compound is doramectin, the active ingredient in DECTOMAX. Therefore claim 1 reads on the approved product.

Claim 2 of U.S. 5,089,460 claims the compounds of claim 1 in which R^4 is restricted to the 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy group. Therefore claim 2 claims doramectin. Therefore claim 2 reads on the approved product.

Claim 17 of U.S. 5,089,480 claims the compounds of claim 2 in which R and R^1 taken together represent a double bond. Therefore claim 17 claims doramectin. Therefore claim 17 reads on the approved product.

Claim 18 of U.S. 5,089,480 claims the compounds of claim 17 in which the meaning of R^2 is restricted to a C_3-C_8 cycloakyl group. Cyclohexyl is a C_6 cycloalkyl group. Therefore claim 18 claims doramectin. Therefore claim 18 reads on the approved product.

Claim 19 of U.S. 5,089,480 claims the compounds of claim 18 in which R^2 is restricted to a cyclohexyl group and R^3 is restricted to hydrogen. Therefore, claim 19 claims doramectin. Therefore claim 19 reads on the approved product.

Claim 33 claims a composition for the treatment and prevention of parasitic infections in animals which contain a compound of claim 1. Since claim 1 claims doramectin, and doramectin has been approved as an injectable parasiticide, claim 33 reads on the approved product.

Claim 34 claims a composition of claim 33 in the form of an injectable formulation. Therefore claim 34 reads on the approved product.

(10) The relevant dates and information pursuant to 35 U.S.C. §156(g) in order to enable the Secretary of Health and Human Services to determine the applicable regulatory review period are as follows.

(a) An exemption under subsection (j) of section 512 of the Federal Food, Drug and Cosmetic Act became effective for DECTOMAX on December 7, 1988 following submission of Investigational New Animal Drug ("INAD") Application No. 6317 on October 21, 1988.[1]

(b) A New Animal Drug Application ("NADA") under section 512 of the Federal Food, Drug and Cosmetic Act for DECTOMAX was initially submitted on March 7, 1996, as NADA No. 141-061.

(c) NADA No. 141-061 was approved on July 30, 1996.

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[1] December 7, 1988 is the date of the first letter received by applicant from FDA (Dr. Gable) which responded substantively to the issues raised in the INAD submission of October 21, 1988. The letter stated that the proposed environmental testing plan was appropriate. Therefore applicant considers that the exemption under subsection (j) of section 512 of the Federal Food Drug and Cosmetic Act became effective on December 7, 1988. A copy of the December 7 letter is attached hereto as Exhibit D.

(11) A brief description of the significant activities undertaken by or for the marketing applicant during the applicable regulatory review period with respect to the approved product and the significant dates applicable to such activities is attached hereto as EXHIBIT E.

(12) Applicant is of the opinion that United States Patent No. 5,089,480 is eligible for an extension under 35 U.S.C. §156, and the length of extension claimed is 527 days.

The eligibility requirements of 35 U.S.C. §156(a) and (c)(4) have been satisfied as follows.

(a) U.S. Patent No. 5,089,480 claims a product, DECTOMAX (doramectin).

(b) U.S. Patent No. 5,089,480 is currently set to expire on February 18, 2009 (i.e., the term of the patent has not yet expired).

(c) The term of U.S. Patent No. 5,089,480 has never been extended under subsection (e)(1) of 35 U.S.C. §156.

(d) This application for extension is being submitted by PFIZER INC., the owner of record of U.S. Patent No. 5,089,480, by its agent, in accordance with the requirements of paragraphs (1) through (4) of 35 U.S.C. §156(d).

(e) The product, DECTOMAX (doramectin), has been subject to a regulatory review period under section 512 of the Federal Food, Drug and Cosmetic Act before its commercial marketing or use, and the permission for said commercial marketing or use is the first permitted commercial marketing or use of the product under section 512 of the Federal Food, Drug and Cosmetic Act.

(f) No patent has to this date been extended, nor has any other extension been applied for, under subsection (e)(1) of 35 U.S.C. §156, for the regulatory review period which forms the basis for this application for extension of the term of U.S Patent No. 5,089,480.

The length of extension of the term of U.S. Patent No. 5,089,480 of 527 days claimed by applicant was determined according to the provisions of 37 C.F.R. §1.778 as follows.

(a) According to 37 C.F.R. §1.778(b), the length of extension is equal to the regulatory review period for the approved product, reduced as appropriate according to paragraphs (d)(1) through (d)(6) of 37 C.F.R. §1.778.

(b) According to 37 C.F.R. §1.778(c), the regulatory review period is the sum of (A) the number of days in the period beginning on the earlier of the date a major health or environmental effects test on the drug was initiated or the date on which an exemption under subsection (j) of section 512 of the Federal Food, Drug and Cosmetic Act became effective and ending on the date the NADA was initially submitted under section 512 of the Federal Food, Drug and Cosmetic Act and (B) the number of days in the period beginning on the date the NADA was initially submitted and ending on the date the NADA was approved. The exemption under subsection (j) of section 512 of the Federal Food, Drug and Cosmetic Act became effective on December 7, 1988; the NADA was initially submitted on March 7, 1996; and the NADA was approved on July 30, 1996. Hence, the regulatory review period under 37 C.F.R. §1.778(c) is the sum of the period from December 7, 1988 to March 7, 1996 and from March 8, 1996 to July 30, 1996. This is the sum of 2,647 days and 145 days, which is 2,792 days.

(c) According to 37 C.F.R. §1.778(d)(1)(i), the number of days in the regulatory review period which were on or before the date on which the patent issued must be subtracted. U.S. Patent No. 5,089,480 issued on February 18, 1992. Subtraction of the period on or before February 18, 1992 leaves a reduced regulatory review period of from February 19, 1992 to March 7, 1996 and from March 8, 1996 to July 30, 1996. This is the sum of 1,479 days and 145 days, which is 1624 days.

(d) 37 C.F.R. §1.778(d)(1)(ii) does not apply.

(e) According to 37 C.F.R. §1.778(d)(1)(iii), the regulatory review period must then be reduced by one-half of the days remaining in the period defined in 37 C.F.R. §1.778(c)(1). This is one-half of 1,479 days, which is 739.5 days. After subtraction, and ignoring half days in the subtraction, this now leaves a reduced regulatory review period of 884 days.

(f) When the reduced regulatory review period of 884 days is added to the expiration date of U.S. Patent No. 5,089,480 (February 18, 2009), this gives a date of July 22, 2011. This latter date is later than July 30, 2010, the date obtained by adding 14 years to the date of approval of the approved product. Therefore, under paragraphs (d)(2) to (d)(4) of 37 C.F.R. §1.778, applicant is entitled to an extension corresponding to the period from February 18, 2009 to July 30, 2010. This is 527 days, which is the length of extension being claimed. Hence, applicant is in compliance with 35 U.S.C. §156(c)(3) and paragraphs (d)(2) to (d)(4) of 37 C.F.R. §1.778.

(g) The five-year limitation of 35 U.S.C. §156(g)(6)(A) and 37 C.F.R. §1.778(d)(5) applies to this application, because U.S. Patent No. 5,089,480 issued after the date of enactment of the Generic Animal Drug and Patent Term Restoration Act (November 16, 1988). When 5 years is added to the expiration date of U.S. Patent No. 5,099,480 (February 18, 2009), this gives a date of February 18, 2014. The date obtained by adding the extension sought (527 days) to the expiration date of U.S. Patent No. 5,089,480 is July 30, 2010, which is earlier than February 18, 2014. Hence, applicant is in compliance with 35 U.S.C. §156(g)(6)(A) and 37 C.F.R. §1.778(d)(5).

(13) Applicant acknowledges a duty to disclose to the Commissioner of Patents and Trademarks and the Secretary of Health and Human Services any information which is material to the determination of entitlement to the 527-day extension being sought to the term of United States Patent No. 5,089,480.

(14) The prescribed fee for receiving and acting on this application for extension is to be charged to Deposit Account No. 16-1445, as requested in the enclosed transmittal letter.

(15) Please address all inquiries and correspondence relating to this application for patent term extension to:

J. Trevor Lumb
Pfizer Inc.
Patent Department
235 East 42nd Street
New York, NY 10017-5755

Tel (212) 573-2521
Fax (212) 573-1939.

(16) A duplicate of these application papers, certified as such, is enclosed herewith.

(17) A declaration pursuant to 37 C.F.R. §§1.740(a)(17) and 1.740(b) is enclosed herewith.

Respectfully submitted,
PFIZER INC.

Date: September 25, 1996

By: J. Trevor Lumb
J. Trevor Lumb, Its Agent
Reg. No. 28,567
Tel.: (212) 573-2521

Pfizer Inc.
Patent Department
235 East 42nd Street
New York, NY 10017-5755

EXHIBIT A

US005089480A

United States Patent [19]

Gibson et al.

[11] Patent Number: 5,089,480

[45] Date of Patent: Feb. 18, 1992

[54] ANTIPARASITIC AGENTS

[75] Inventors: Stephen P. Gibson, Westbrook; Alexander C. Goudie; Kelvin S. Holdom, both of Ramsgate; John D. Bu'lock, Manchester, all of England

[73] Assignee: Pfizer Inc., New York, N.Y.

[21] Appl. No.: 142,888

[22] Filed: Jan. 11, 1988

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 886,867, Jul. 16, 1986, abandoned.

[30] Foreign Application Priority Data

Jul. 27, 1985	[GB]	United Kingdom	8518999
Aug. 9, 1985	[GB]	United Kingdom	8520069
Apr. 24, 1986	[GB]	United Kingdom	8610063
May 2, 1986	[GB]	United Kingdom	8610862

[51] Int. Cl.[5] A61K 31/71; C07H 17/08; C07D 493/20

[52] U.S. Cl. 514/30; 514/450; 536/77; 549/264

[58] Field of Search 514/30, 450; 536/7.1; 549/264

[56] References Cited

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Cane, D. E. et al., J. Am. Chem. Soc. 105:4110-4112 (1983).
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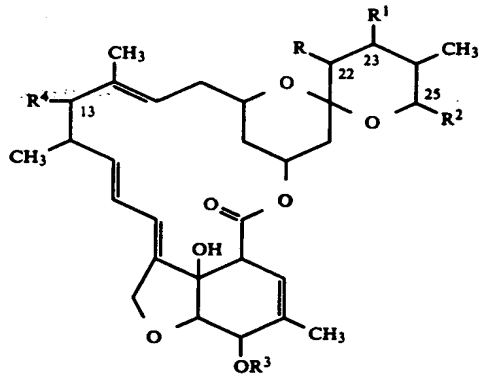
(List continued on next page.)

Primary Examiner—Johnnie R. Brown
Assistant Examiner—Elli Peselev
Attorney, Agent, or Firm—Peter C. Richardson; J. Trevor Lumb; Gregg C. Benson

[57] ABSTRACT

The invention provides novel compounds having the formula:



(I)

wherein R when taken individually is H; R^1 when taken individually is H or OH; R and R^1 when taken together represent a double bond;

R^2 is an alpha-branched C_3-C_8 alkyl, alkenyl, alkynyl, alkoxyalkyl or alkylthioalkyl group; a C_3-C_8 cycloalkyl, C_5-C_8 cycloalkenyl or C_5-C_8 cycloalkylalkyl group, any of which may be substituted by methylene or one or more C_1-C_4 alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be substituted by one or more C_1-C_4 alkyl groups or halo atoms;

R^3 is hydrogen or methyl;

R^4 is H or 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy with the proviso that when R^2 is alkyl it is not isopropyl or sec-butyl; when R^4 is H, each of R and R^1 is H, and R^2 is not methyl or ethyl; and when R^4 is H, R is H, R^1 is OH, and R^2 is not 2-buten-2-yl, 2-penten-2-yl or 4-methyl-2-penten-2-yl.

The compounds are broad spectrum antiparasitic agents having utility as anthelmintics, ectoparasiticides, insecticides and acaricides. The invention also provides a process for producing the novel avermectin and milbemycin derivatives by adding a carboxylic acid or derivative thereof to a fermentation of an avermectin or milbemycin producing organism.

36 Claims, No Drawings

OTHER PUBLICATIONS

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ANTIPARASITIC AGENTS

CROSS-REFERENCE TO RELATED APPLICATION

This application is a continuation-in-part of application Ser. No. 886,867, filed July 16, 1986, now abandoned.

BACKGROUND OF THE INVENTION

1. Field of the Invention

This invention relates to antiparasitic agents and in particular to compounds related to the avermectins and milbemycins but having a novel substituent group at the 25-position and to a process for their preparation.

2. Description of the Prior Art

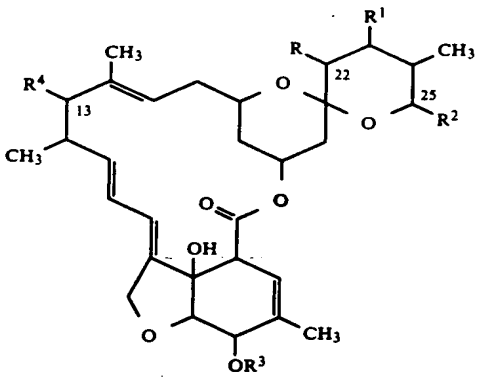
The avermectins are a group of broad spectrum antiparasitic agents referred to previously as the C-076 compounds. They are produced by fermenting a strain of the microorganism *Streptomyces avermitilis* ATCC 31267, 31271 or 31272 under aerobic conditions in an aqueous nutrient medium containing inorganic salts and assimilable sources of carbon and nitrogen. The morphological and cultural properties of the strains ATCC 31267, 31271 and 31272 are described in detail in British Patent Specification No. 1573955 which also describes the isolation and the chemical structure of the eight individual components which make up the C-076 complex. The milbemycins are structurally related macrolide antibiotics lacking the sugar residues at the 13-position. They are produced by fermentation, for example as described in British Patent Specification No. 1390336 and European Patent Application Publication No. 0170006.

SUMMARY OF THE INVENTION

We have now discovered that by adding certain specified carboxylic acids, or derivatives thereof, to the fermentation of an avermectin producing organism it is possible to obtain novel compounds, related to the avermectins but having an unnatural substituent group at the 25-position in place of the isopropyl or sec-butyl group which is normally present. The novel compounds are highly active antiparasitic agents having particular utility as anthelmintics, ectoparasiticides, insecticides and acaricides.

Thus, according to one aspect of the invention there is provided a process for producing a novel avermectin derivative having an unnatural substituent group at the 25-position which comprises adding a carboxylic acid, or a salt, ester or amide thereof or oxidative precursor therefor, to a fermentation of an avermectin producing organism, and isolating the novel avermectin derivative.

Conventional chemical transformation reactions can be used to prepare further derivatives from these compounds. Thus, according to a further aspect of the invention there are provided compounds having the formula:



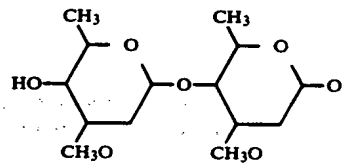
(I)

wherein R when taken individually is H; R^1 when taken individually is H or OH; R and R^1 when taken together represent a double bond;

R^2 is an alpha-branched C_3–C_8 alkyl, alkenyl, alkynyl, alkoxyalkyl or alkylthioalkyl group; a C_5–C_8 cycloalkylalkyl group wherein the alkyl group is an alpha-branched C_2–C_5 alkyl group; a C_3–C_8 cycloalkyl or C_5–C_8 cycloalkenyl group, either of which may be substituted by methylene or one or more C_1–C_4 alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may be substituted by one or more C_1–C_4 alkyl groups or halo atoms;

R^3 is hydrogen or methyl;

R^4 is H or a 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy group of the formula:



with the proviso that when R^2 is alkyl it is not isopropyl or sec-butyl; when R^4 is H, each of R and R^1 is H, and R^2 is not methyl or ethyl; and when R^4 is H, R is H, R^1 is OH, and R^2 is not 2-buten-2-yl, 2-penten-2-yl or 4-methyl-2-penten-2-yl.

In the above definition, alkyl groups containing 3 or more carbon atoms may be straight or branched chain. Halo means fluoro, chloro, bromo or iodo. Alpha-branched means that the carbon atom attached to the 25-ring position is a secondary carbon atom linked to two further carbon atoms. When R^2 is alkyl of 5 or more carbon atoms, the remainder of the alkyl chain may be straight or branched chain.

Preferred compounds of the formula I are those wherein R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy. Also preferred are compounds of the formula I wherein R^2 is a C_5 or C_6 cycloalkyl or cycloalkenyl group which may be substituted by one or more C_1–C_4 alkyl groups, cyclopentyl and cyclohexyl being particularly preferred. In another group of preferred compounds R^2 is cyclobutyl. In a further group of preferred compounds R^2 is a 5 or 6 membered oxygen or sulphur containing heterocyclic ring, particularly a 3-thienyl or 3-furyl ring, which may be substituted by

one or more C_1–C_4 alkyl groups or halogen atoms. In a yet further group of preferred compounds, R^2 is a C_3–C_8 alkylthioalkyl group, particularly a 1-methylthioethyl group.

DETAILED DESCRIPTION OF THE INVENTION

In accordance with the invention the compounds of formula I wherein R is H and R^1 is OH or wherein R and R^1 taken together represent a double bond, and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy are prepared by fermenting an avermectin producing organism, such as a strain of the organism *Streptomyces avermitilis* ATCC 31267, 31271 or 31272, in the presence of the appropriate carboxylic acid of the formula R^2CO_2H, wherein R^2 is as previously defined, or a salt, ester, or amide thereof or oxidative precursor therefor. The acid is added to the fermentation either at the time of inoculation or at intervals during the fermentation. Production of the compounds of formula (I) may be monitored by removing samples from the fermentation, extracting with an organic solvent and following the appearance of the compound of formula (I) by chromatography, for example using high pressure liquid chromatography. Incubation is continued until the yield of the compound of formula (I) has been maximised, generally for a period of from 4 to 6 days.

A preferred level of each addition of the carboxylic acid or derivative thereof is between 0.05 and 1.0 grams per liter. The best yields of the compounds of formula (I) are obtained by gradually adding the acid to the fermentation, for example by daily additions of the acid or derivative thereof over a period of several days. The acid is preferably added as a salt, such as the sodium or ammonium salt, but may be added as an ester, such as the methyl or ethyl ester or as an amide. Alternative substrates which may be used in the fermentation are derivatives which are oxidative precursors for the carboxylic acids; thus, for example suitable substrates would be aminoacids of the formula $R^2CH(NH_2)CO_2H$, glyoxylic acids of the formula R^2COCO_2H, methylamine derivatives of the formula $R^2CH_2NH_2$, substituted lower alkanoic acids of the formula $R^2(CH_2)_nCO_2H$ wherein n is 2, 4 or 6, methanol derivatives of the formula R^2CH_2OH or aldehydes of the formula R^2CHO, wherein R^2 is as previously defined. The media used for the fermentation may be a conventional complex media containing assimilable sources of carbon, nitrogen and other trace elements. However we have found that for better results a strain of the organism derived from *Streptomyces avermitilis* ATCC 31271 which gives improved yields of a compound of formula I when cultured in a semi-defined medium may be used and this has the advantage that crude solvent extracts contain significantly less unwanted material which greatly simplifies the subsequent isolation and purification stages. Such a strain has been deposited with the National Collection of Industrial Bacteria (NCIB) on 19th July, 1985 under the accession number NCIB 12121. The morphological and cultural characteristics of this strain are otherwise generally as described in British Patent specification No. 1573955 for strain ATCC 31267.

After fermentation for a period of several days at a temperature preferably in the range of from 24° to 33° C., the fermentation broth is centrifuged or filtered and the mycelial cake is extracted with acetone or methanol. The solvent extract is concentrated and the desired product is then extracted into a water-immiscible organic solvent, such as methylene chloride, ethyl acetate, chloroform, butanol or methyl isobutyl ketone. The solvent extract is concentrated and the crude product containing the compounds of formula (I) is further purified as necessary by chromatography, for example using preparative reverse phase, high pressure liquid chromatography.

The product is generally obtained as a mixture of the compounds of formula (I) wherein R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy, R is H, R^1 is OH or R and R^1 taken together represent a double bond and wherein R^3 is H or CH_3; however the proportions can vary depending on the particular carboxylic acid employed and the conditions used.

We have found that a broad range of carboxylic acids as defined by R^2CO_2H may be added to the fermentation to yield avermectins having a novel substituent group at the 25-position. Examples of particular acids which may be employed include the following:

2-methylvaleric acid
2-methylpent-4-enoic acid
2-methylthiopropionic acid
2-cyclopropyl propionic acid
cyclobutane carboxylic acid
cyclopentane carboxylic acid
cyclohexane carboxylic acid
cycloheptane carboxylic acid
2-methylcyclopropane carboxylic acid
3-cyclohexene-1-carboxylic acid and
thiophene-3-carboxylic acid

In one particular and preferred aspect of the invention, the fermentation is performed in the presence of cyclopentane carboxylic acid sodium salt to yield predominantly the compound of formula (I) wherein R is H, R^1 is OH, R^2 is cyclopentyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy.

In another preferred aspect of the invention, the fermentation is performed in the presence of thiophene-3-carboxylic acid sodium salt to yield predominantly the compound of (I) where R is H, R^1 is OH, R^2 is thien-3-yl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)alpha-L-oleandrosyloxy.

In a further preferred aspect of the invention the fermentation is performed in the presence of 2-methylthiopropionic acid sodium salt to yield predominantly the compound of formula (I) wherein R is H, R^1 is OH, R^2 is 1-methylthioethyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy.

Compounds of the formula (I) wherein the C_{22-23} double bond is present may alternatively be prepared from the corresponding compound of formula (I) wherein R is H and R^1 is OH by a dehydration reaction. The reaction is performed by first selectively protecting the hydroxyl groups at the 5 and 4'' positions, e.g. as the t-butyldimethylsilyloxy acetyl derivative, then reacting with a substituted thiocarbonyl halide, such as (4-methylphenoxy)thiocarbonyl chloride, followed by heating in a high boiling point solvent, e.g. trichlorobenzene, to effect the dehydration. The product is finally deprotected to give the unsaturated compound. These steps together with appropriate reagents and reaction conditions are described in U.S. Pat. No. 4,328,335.

The compounds of formula I wherein R^3 is H may also be prepared from the corresponding compounds wherein R^3 is CH_3 by demethylation. This reaction is achieved by treating the 5-methoxy compound, or a

suitably protected derivative thereof, with mercuric acetate and hydrolysing the resulting 3-acetoxy enol ether with dilute acid to give the 5-keto compound. This is then reduced using, for example, sodium borohydride to yield the 5-hydroxy derivative. Appropriate reagents and reaction conditions for these steps are described in U.S. Pat. No. 4,423,209.

The compounds of formula I wherein each of R and R^1 is H can be prepared from the corresponding compound wherein the double bond is present at C_{22}–C_{23} by selective catalytic hydrogenation using an appropriate catalyst. For example the reduction may be achieved using tris(triphenylphosphine)rhodium (I) chloride as described in European patent application publication No. 0001689.

The compounds of formula (I) wherein R^4 is H are prepared from the corresponding compounds wherein R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy by removing the 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrose group by mild hydrolysis with an acid in an aqueous organic solvent to yield the aglycone having a hydroxy group at the 13-position; this is then halogenated, for example by reaction with a benzene sulphonyl halide, to yield the 13-deoxy-13-halo derivative which is finally selectively reduced, for example using tributyltin hydride. In order to avoid unwanted side reactions it is desirable to protect any other hydroxy groups which may be present, for example using a tert-butyldimethylsilyl group. This is then readily removed after the halogenation or reduction step by treatment with methanol containing a trace of acid. All these steps together with appropriate reagents and reaction conditions for their performance are described in European patent application publication No. 0002615.

Compounds of the formula (I) wherein each of R and R^4 is H and R^1 is either H or OH, may also be prepared by adding the appropriate carboxylic acid, or a salt, ester or amide thereof or oxidative precursor therefor, to a fermentation of a milbemycin producing organism, and isolating the desired milbemycin derivative having an unnatural substituent group at the 25-position. Examples of milbemycin producing organisms include for instance *Streptomyces hygroscopicus* strain NRRL 5739 as described in British Patent Specification No. 1390336, *Streptomyces cyaneogriseus* subsp. noncyanogenus NRRL 15773 as described in European patent application publication No. 0170006 and Streptomyces thermoarchaenis NCIB 12015 as described in GB 2166436A.

The compounds of the invention are highly active antiparasitic agents having particular utility as anthelmintics, ectoparasiticides, insecticides and acaricides.

Thus the compounds are effective in treating and preventing a variety of conditions caused by endoparasites including, in particular, helminthiasis which is most frequently caused by a group of parasitic worms described as nematodes and which can cause severe economic losses in swine, sheep, horses and cattle as well as affecting domestic animals and poultry. The compounds are also effective against other nematodes which affect various species of animals including, for example, Dirofilaria in dogs and various parasites such as Ancylostoma, Necator, Ascaris, Strongyloides, Trichinella, Capillaria, Trichuris, Enterobius and parasites which are found in the blood or other tissues and organs such as filiarial worms and the extra intestinal stages of Strongyloides and Trichinella.

The compounds are also of value in treating and preventing ectoparasite infections including in particular arthropod ectoparasites of animals and birds such as ticks, mites, lice, fleas, blowfly, biting insects and migrating dipterous larvae which can affect cattle and horses.

The compounds are also insecticides active against household pests such as the cockroach, clothes moth, carpet beetle and the housefly as well as being useful against insect pests of stored grain and of agricultural plants such as spider mites, aphids, caterpillars and against migratory orthopterans such as locusts.

The compounds of formula (I) are administered as a formulation appropriate to the specific use envisaged and to the particular species of host animal being treated and the parasite or insect involved. For use as an anthelmintic the compounds may be administered orally in the form of a capsule, bolus, tablet or preferably a liquid drench, or alternatively, they may be administered by injection or as an implant. Such formulations are prepared in a conventional manner in accordance with standard veterinary practice. Thus, capsules, boluses or tablets may be prepared by mixing the active ingredient with a suitable finely divided diluent or carrier additionally containing a disintegrating agent and/or binder such as starch, lactose, talc, magnesium stearate, etc. A drench formulation may be prepared by dispersing the active ingredient in an aqueous solution together with dispersing or wetting agents etc. and injectable formulations may be prepared in the form of a sterile solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. These formulations will vary with regard to the weight of active compound depending on the species of host animal to be treated, the severity and type of infection and the body weight of the host. Generally for oral administration a dose of from about 0.001 to 10 mg per Kg of animal body weight given as a single dose or in divided doses for a period of from 1 to 5 days will be satisfactory but of course there can be instances where higher or lower dosage ranges are indicated and such are within the scope of this invention.

As an alternative the compounds may be administered with the animal feedstuff and for this purpose a concentrated feed additive or premix may be prepared for mixing with the normal animal feed.

For use as an insecticide and for treating agricultural pests the compounds are applied as sprays, dusts, emulsions and the like in accordance with standard agricultural practice.

The invention is illustrated by the following Examples in which Examples 1 to 21 are Examples of the preparation of compounds of the formula (I), Example 22 is an example of a drench formulation and Examples 23 and 24 illustrate the antiparasitic and insecticidal activity of the compounds.

EXAMPLE 1

25-Cyclopentyl-avermectin A2

A suspension of a slope culture of *S. avermitilis* NCIB 12121 was inoculated into 600 ml of a medium containing lactose (12.0 g), distillers solubles (8.0 g) and yeast extract (3.0 g), contained in a 3 liter flask, and incubated at 28° C. for 3 days. The inoculum was used to inoculate 16 liters of a medium containing soluble starch (640 g), ammonium sulphate (32 g), dipotassium hydrogen phosphate (16 g), sodium chloride (16 g), magnesium sul-

phate $7H_2O$ (16 g), calcium carbonate (32 g), soluble yeast extract (6.4 g), ferrous sulphate $7H_2O$ (0.016 g), zinc sulphate $7H_2O$ (0.016 g) and manganese chloride $4H_2O$ (0.016 g), contained in a 20 liter fermenter. The fermentation was incubated at 28° C., with agitation at 250 r.p.m. and aerated at 15 liters per minute. Cyclopentane carboxylic acid sodium salt (1.6 g) was added after 24 hours and again after 48 and 72 hours incubation and the fermentation was continued for 120 hours. After this time the mycelium was removed by filtration and extracted with acetone:1N-hydrochloric acid (100:1; 3×7 liters). The extract was concentrated to approximately 2 liters under reduced pressure and extracted with methylene chloride (2×5 liters). The methylene chloride extract was concentrated to dryness to give the crude product as a mobile oil which was dissolved in diethyl ether and added to a column of silica gel (1 kg). The column was eluted with diethyl ether collecting 100 ml fractions. Fractions 20–40 were combined and the solvent evaporated to yield partially purified material. The product was dissolved in a mixture of methanol and water (4:1) and chromatographed on a C_{18} Micro-Bondapack column (50 mm×50 cm) in a Waters Prep 500 high pressure liquid chromatograph using the same solvent at a flow rate of 100 ml per minute. Fractions 35 to 50 containing the desired product were combined and rechromatographed on a C_{18} Zorbax ODS (Trademark, Dupont) column (21 mm×25 cm) eluting with a mixture of methanol and water (4:1) at a flow rate of 9 ml per minute. The relevant fractions were combined and the solvent evaporated to yield the compound of formula (I) wherein R is H, R^1 is OH, R^2 is cyclopentyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy as a white powder, m.p. 150.5°–151° C. The structure of the product was confirmed by mass spectrometry and by C_{13} nuclear magnetic resonance spectroscopy as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M+Na)^+$ observed at m/e 939 (theoretical 939).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 335, 317, 275, 257, 251, 233, 205, 181, 179, 145, 127, 113, 111, 95 and 87.

The 13C nuclear magnetic resonance spectral data were obtained on a Brucker Model WM-250 spectrometer with a sample concentration of 20 mg/ml in deuterochloroform. The chemical shifts in parts per million relative to tetramethylsilane were: 14.1, 15.3, 17.8, 18.5, 19.9, 20.3, 24.6 25.9, 26.2, 29.3, 34.4 (2C), 34.7, 36.7, 37.8, 39.8, 40.5, 41.0, 41.3, 45.8, 56.4, 56.6, 57.8, 67.4, 67.6, 68.0, 68.3, 68.7, 69.9, 70.5, 76.0, 77.6 (2C), 78.3, 79.5, 80.7 (2C), 81.8, 94.9, 98.7, 99.8, 117.7, 118.5, 119.8, 125.0, 135.8, 136.3, 137.8, 140.1 and 173.8.

EXAMPLE 2

A suspension of a slope culture of *S. avermitilis* ATCC 31271 was inoculated into 50 ml of a medium containing lactose (1.0 g), distillers solubles (0.75 g) and yeast extract (0.25 g), contained in a 350 ml flask, and incubated at 28° C. for 3 days. This inoculum 4 ml) was used to inoculate each of 50 flasks containing 50 ml of medium containing corn starch (2.0 g), soya flour (0.35 g) and yeast extract (0.25 g) contained in a 350 ml flask, and the flasks were incubated at 28° C.

After 24 hours, cyclopentane carboxylic acid sodium salt (5 mg) was added to each flask and incubation was continued for a further 5 days. After this time the contents of the flasks were bulked and the mycelium separated by centrifugation. The mycelium was extracted with acetone: 1N-hydrochloric acid (100:1) and the acetone extract concentrated to dryness. The extract was analysed by high pressure liquid chromatography and was shown to contain a product identical with the product of Example 1.

EXAMPLE 3

An inoculum was prepared as described in Example 1 and used to inoculate 50 ml of the medium as used in Example 1, contained in 350 ml flasks. After incubation for 24 hours, 2-aminocyclopentyl acetic acid (cyclopentylglycine) (5 mg) was added and the fermentation was continued for a further 5 days. The product was recovered by extraction of the mycelium with acetone and methylene chloride. The extract was analyzed by HPLC which indicated that the product contained a compound identical to the product of Example 1.

EXAMPLE 4

The conditions of Example 3 were followed except that cyclopentyl methanol was used as substrate with similar results.

EXAMPLE 5

The conditions of Example 3 were followed except that the methyl ester of cyclopentane carboxylic acid, dissolved in methanol, was used as substrate with similar results.

EXAMPLE 6

The conditions of Example 3 were followed except that cyclopentane carboxylic acid, dissolved in methanol was used as substrate with similar results.

EXAMPLE 7

25-(Thien-3-yl)avermectin

A suspension of a slope culture of *S. avermitilis* NCIB 12121 was inoculated into 600 ml of a medium containing lactose (12.0 g), distillers solubles (8.0 g) and yeast extract (3.0 g), contained in a 3 liter flask, and incubated at 28° C. for 3 days. The inoculum was used to inoculate 16 liters of a medium containing soluble starch (640 g), ammonium sulphate (32 g), dipotassium hydrogen phosphate (16 g), sodium chloride (16 g), magnesium sulphate $7H_2O$ (16 g), calcium carbonate (32 g), soluble yeast extract (6.4 g), ferrous sulphate $7H_2O$ (0.016 g), zinc sulphate $7H_2O$ (0.016 g) and manganese chloride $4H_2O$ (0.016 g), contained in a 20 liter fermenter. The fermentation was incubated at 28° C., with agitation at 250 r.p.m. and aerated at 15 liters per minute. Thiophene-3-carboxylic acid sodium salt (1.6 g) was added after 24 hours and again after 48 and 72 hours incubation and the fermentation was continued for 120 hours. After this time the mycelium was removed by filtration and extracted with acetone:1N-hydrochloric acid (100:1; 3×7 liters). The extract was concentrated to approximately 2 liters under reduced pressure and extracted with methylene chloride (2×5 liters). The methylene chloride extract was concentrated to dryness to give the crude product as a mobile oil which was dissolved in diethyl ether and added to a column of silica gel (1 kg). The column was eluted with diethyl

ether collecting 200 ml fractions. Fractions 32–45 were combined and the solvent evaporated to yield partially purified material. The product was dissolved in a mixture of methanol and water (3:1) and chromatographed on a C_{18} Micro-Bondapack column (50 mm×50 cm) in a Waters Prep 500 high pressure liquid chromatograph using the same solvent at a flow rate of 100 ml per minute. Fractions 27 to 36 containing the desired product were combined and rechromatographed on a C_{18} Zorbax ODS (Trademark, Dupont) column (21 mm×25 cm) eluting with a mixture of methanol and water (3:1) at a flow rate of 9 ml per minute. The relevant fractions were combined and the solvent evaporated to yield the compound of formula (I) wherein R is H, R^1 is OH, R^2 is thien-3-yl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy as a white powder, m.p. 167° C. The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M+Na)^+$ observed at m/e 953 (theoretical 953).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 349, 331, 275, 265, 257, 247, 237, 219, 195, 145, 127, 113, 95 and 87.

EXAMPLE 8

A vegetative cell suspension of *S. avermitilis* NCIB 12121, held at −60° C. in 10% v/v aqueous (2 ml) glycerol was inoculated into 50 ml of medium containing lactose (1.0 g), distillers solubles (0.75 g) and yeast extract (0.25 g) contained in a 300 ml conical flask and incubated at 28° C. for 24 hours, with shaking. The inoculum was then added to 600 ml of the above medium contained in a 3 liter flask and the mixture was incubated at 28° C. for 24 hours with shaking. The product was used to inoculate 10 liters of the above medium contained in a 16 liter fermenter which was incubated at 28° C. for 24 hours at an agitation speed of 350 r.p.m. with aeration at 10 liters of air per minute. This fermentation (600 ml) was used to inoculate 16 liters of a medium containing partially hydrolysed starch (640 g) ammonium sulphate (32 g), dipotassium hydrogen phosphate (16 g), sodium chloride (16 g) magnesium sulphate $7H_2O$ (16 g), calcium carbonate (32 g), soluble yeast extract (6.4 g), ferrous sulphate $7H_2O$ (0.016 g), zinc sulphate $7H_2O$ (0.016 g), and manganese chloride $4H_2O$ (0.016 g), contained in a 20 liter fermenter. The fermentation was incubated at 28° C., with agitation at 350 r.p.m. and aerated at 15 liters per minute. Cyclobutane carboxylic acid sodium salt (1.6 g) was added after 24 hours and again after 48 and 72 hours incubation and the fermentation was continued for 120 hours. After this time the mycelium was removed by filtration and extracted with acetone (3×7 liters). The extract was concentrated to approximately 2 liters under reduced pressure and extracted with methylene chloride (2×5 liters). The methylene chloride was concentrated to dryness to give the crude product as a mobile oil. This was taken up in iso-octane (150 ml) and the solution extracted with a mixture of methanol (95 ml) and water (5 ml). Evaporation of the methanolic extract gave partially purified material which was separated into its individual components by high pressure liquid chromatography as follows: The residue was dissolved in a little methanol and chromatographed in a C_{18} Micro-Bondapack column (50 mm×50 cm) in a Waters Prep 500 high pressure liquid chromatograph using a mixture of methanol/water (4:1) at a flow rate of 100 ml per minute. Fractions 1 to 4 were combined and used in Example 9, fractions 5 to 9 were combined and used in Example 10, fractions 10 to 19 were combined and used in Example 11 and fractions 20 to 35 were combined and used in Example 12.

EXAMPLE 9

25-Cyclobutyl-avermectin B2 (R^1=OH, R and R^3=H)

The combined fractions 1 to 4 from Example 8 were evaporated to dryness and the residue was rechromatographed on a C_{18} Zorbax ODS (Trademark, Dupont) column (21 mm×25 cm) eluting with a mixture of methanol and water (3:1) at a flow rate of 9 ml per minute. The relevant fractions were combined, the solvent evaporated and the product subjected to a final purification on a Silica Spherisorb 5 micron (Trademark, HPLC Technology) column (10.5 mm×25 cm) eluting with a mixture of methylene chloride and methanol (98:2) at a flow rate of 4 ml per minute. The relevant fractions were combined and the solvent evaporated to yield the compound of formula (I) wherein R is H, R^1 is OH, R^2 is cyclobutyl, R^3 is H and R^4 is 4'-(alpha-L-oleandrosyl)-L-oleandrosyloxy, as a white powder, m.p. 110°–112° C. The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M+Na)^+$ observed at m/e 911 (theoretical 911).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 321, 303, 261, 257, 237, 219, 209, 191, 179, 167, 145, 127, 113, 111, 95 and 87.

EXAMPLE 10

25-Cyclobutyl-avermectin A2 (R^1=OH, R=H, $R^3=CH_3$)

The combined fractions 5 to 9 from Example 8 were evaporated to dryness and the residue was rechromatographed twice on a C_{18} Zorbax ODS (Trademark, Dupont) column, (21 mm×25 cm) eluting with a methanol and water mixture (77:23) at a flow rate of 9 ml per minute. Suitable fractions were combined and evaporated to yield the compound of formula (I) wherein R is H, R^1 is OH, R^2 is cyclobutyl, R^3 is CH_2 and R^4 is 4'-(alpha-L-oleandrosyl)-L-oleandrosyloxy, as a white powder, m.p. 135°–140° C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M+Na)^+$ observed at m/e 925 (theoretical 925).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 596, 454, 321, 303, 275, 237, 219, 209, 191, 179, 167, 145, 127, 113, 111, 95 and 87.

EXAMPLE 11

25-Cyclobutyl-avermectin B1

(R and R^1 taken together = Double bond, R^3 = H)

The combined fractions 10 to 19 from Example 8 were evaporated to dryness and the residue dissolved in methanol and chromatographed on a C_{18} Zorbax ODS (Trademark, Dupont) column, (21 mm × 25 cm) eluting with a mixture of methanol and water (4:1) at a flow rate of 9 ml per minute. The relevant fractions were combined and the solvent evaporated to give a product which was rechromatographed on a Silica Zorbax SIL (Trademark, Dupont) column (21 mm × 25 cm) eluting with a mixture of dichloromethane and methanol (98.5:1.5) at a flow rate of 9 ml per minute. The relevant fractions were combined and the solvent evaporated to yield the compound of formula (I) wherein R and R^1 taken together represent a double bond, R^2 is cyclobutyl, R^3 is H and R^4 is 4'-(alpha-L-oleadrosyl)-L-oleandrosyloxy, as a white powder, m.p. 135°–138° C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M+Na)^+$ observed at m/e 893 (theoretical 893).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 303, 261, 257, 219, 191, 167, 145, 127, 113, 111, 95 and 87.

EXAMPLE 12

25-Cyclobutyl-avermectin A1

(R and R^1 taken together = Double bond, $R^3 = CH_3$)

The combined fractions 20 to 35 from Example 8 were evaporated to dryness and the residue chromatographed on a C_{18} Zorbax ODS (Trademark, Dupont) column (21 mm × 25 cm) at a flow rate of 9 ml per minute. The relevant fractions were combined, the solvent evaporated and the product was rechromatographed on a Silica Sperisorb 5 micron (Trademark, HPLC Technology) column (10.5 mm × 25 cm) eluting with a mixture of dichloromethane and methanol (98.5:1.5) at a flow rate of 4 ml per minute. Combination of the relevant fractions followed by evaporation gave the compound of formula (I) wherein R and R^1 taken together represent a double bond, R^2 is cyclobutyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-L-oleandrosyloxy, as a white powder, m.p. 120°–124° C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M+Na)^+$ observed at m/e 907 (theoretical 907).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 578, 303, 275, 257, 219, 191, 167, 145, 127, 113, 111, 95 and 87.

EXAMPLE 13

25-(Cyclohex-3-enyl)avermectin A2

The medium and conditions of Example 1 were followed except that 3-cyclohexenoic acid sodium salt was used as the substrate to yield the compound of formula I wherein R is H, R^1 is OH, R^2 is cyclohex-3-enyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy, as a white powder, m.p. 131°–5° C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M+Na)^+$ observed at m/e 951 (theoretical 951).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 624, 480, 347, 329, 275, 245, 235, 217, 205, 193, 179, 145, 127, 113, 111, 95 and 87.

EXAMPLE 14

25-Cyclohexyl avermectin A2

The medium and conditions of Example 1 were followed except that cyclohexane carboxylic acid sodium salt was used as the substrate to yield the compound of formula I wherein R is H, R^1 is OH, R^2 is cyclohexyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy, as a white powder, m.p. 112°–117° C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M+Na)^+$ observed at m/e 953 (theoretical 953).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 624, 482, 349, 331, 275, 265, 247, 237, 219, 207, 195, 179, 145, 127, 113, 111, 95 and 87.

EXAMPLE 15

25-(1-Methylthioethyl) avermectin A2

The medium and conditions of Example 1 were followed except that 2-methylthiopropionic acid sodium salt was used as the substrate to yield the compound of formula I wherein R is H, R^1 is OH, R^2 is 1-methylthioethyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-oleandrosyl)-L-oleandrosyloxy, as a white powder, m.p. 134°–138° C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M+Na)^+$ observed at m/e 945 (theoretical 945).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 341, 323, 275, 263, 257, 239, 211, 187, 179, 145, 127, 113, 111, 95 and 87.

EXAMPLE 16

25-(2-Methylcyclopropyl) avermectin A2

The medium and conditions of Example 1 were followed except that 2-methylcyclopropane carboxylic acid sodium salt was used as the substrate to yield the compound of formula I wherein R is H, R^1 is OH, R^2 is 2-methylcyclopropyl, R^3 is CH_3 and R^4 is 4'-(alpha-L-

oleandrosyl)-L-oleandrosyloxy, as a white powder, m.p. 147°–150° C.

The structure of the product was confirmed by mass spectrometry as follows:

Fast atom bombardment mass spectrometry was performed on a VG Model 7070E mass spectrometer using a sample matrix of triethylene glycol with solid sodium chloride. $(M+Na)^+$ observed at m/e 925 (theoretical 925).

Electron impact mass spectrometry was performed using a VG Model 7070F mass spectrometer. The m/e values for the principal fragments were: 596, 454, 303, 275, 237, 219, 209, 191, 179, 167, 145, 127, 113, 111, 95 and 87.

EXAMPLE 17

The procedure of Example 1 was followed but using the sodium salt of the following carboxylic acids as substrate instead of cyclopentane carboxylic acid to yield the appropriate 25-substituted avermectins of formula (I) wherein R is H, R^1 is OH, or R and R^1 taken together represent a double bond, R^3 is H or OH and R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy:

2-methylvaleric acid
2,3-dimethylbutyric acid
2-methylhexanoic acid
2-methylpent-4-enoic acid
2-cyclopropyl propionic acid
cycloheptane carboxylic acid
4,4-difluorocyclohexane carboxylic acid
4-methylenecyclohexane carboxylic acid
3-methylcyclohexane carboxylic acid
cyclopentene-1-carboxylic acid
1-cyclohexene carboxylic acid
tetrahydropyran-4-carboxylic acid
3-furoic acid and
2-chloro-thiophene-4-carboxylic acid.

EXAMPLE 18

Repetition of the procedure of Example 17 but using the carboxylic acids (as their sodium salts) enumerated below, the appropriate 25-substituted avermectins characterized in Table I were obtained:

cyclohexane carboxylic acid
cyclohex-3-ene carboxylic acid
cyclopentane carboxylic acid
2-methylpent-3-enoic acid
2-methylpropionic acid
thiophene-3-carboxylic acid
exomethylenecyclohexane carboxylic acid
furan-3-carboxylic acid
2-methylvaleric acid
thiophene-2-carboxylic acid
tetrahydropyran-4-carboxylic acid
2-methyl-4-methoxybutyric acid
2-methylpent-3-ynoic acid
cyclopent-3-ene carboxylic acid
3,4-dihydropyran-2-carboxylic acid.

TABLE 1

Physical and Spectroscopic Data for Novel C-25 Avermectins

25 Substituent (R^2)	Sub-class	m.p. °C.	Theoretical Mol. Wt.	$(M + Na)^+$ From FAB Mass Spec.	m/e for Principle Fragments in the EI Mass. Spec.
Cyclohexyl	A1	110–115	912	935	606, 331, 275, 257, 247, 218, 195, 145, 127, 113, 95 and 87.
	B1	116–9	898	921	592, 331, 257, 247, 219, 195, 145, 127, 113, 95 and 87.
	B2	146–8	916	939	610, 482, 349, 331, 275, 265, 257, 179, 145, 127, 113, 95 and 87.
	H_2B1^a	150 (dec)	900	923	594, 333, 249, 221, 145, 127, 113, 95 and 87.
3-Cyclohexenyl	B1	122–129	896	919	590, 329, 257, 245, 217, 193, 145, 127, 113, 95 and 87.
Cyclopentyl	B1	158–162	884	907	578, 468, 317, 257, 233, 205, 145, 127, 113, 95 and 87.
	B2	158–164	902	925	596, 468, 335, 317, 257, 251, 233, 179, 145, 127, 113, 95 and 87.
	H_2B1^a	145–147	886	909	580, 319, 257, 207, 145, 127, 113, 95 and 87.
1-Methylbut-3-enyl	A2	149–151	916	939	610, 335, 317, 275, 251, 233, 223, 205, 179, 145, 127, 113, 95 and 87.
	B1	141–144	884	907	596, 578, 317, 261, 257, 233, 205, 145, 127, 113, 95 and 87.
1-Methylthioethyl	B1	144–147	890	913	584, 323, 261, 257, 239, 211, 187, 145, 127, 113, 95 and 87.
3-Thienyl	B1	155–165	898	921	610, 592, 574, 482, 331, 261, 257, 247, 219, 195, 145, 127, 113, 95 and 87.
	B2	175–180	916	939	610, 331, 257, 249, 234, 219, 179, 145, 127, 113, 95 and 87.
Exomethylene-cyclohexyl	B1	161–165	910	933	604, 343, 261, 259, 231, 207, 145, 127, 113, 95 and 87.
3-Furanyl	A2	148–153	914	937	333, 315, 275, 257, 249, 231, 221, 203, 179, 145,

TABLE 1-continued

Physical and Spectroscopic Data for Novel C-25 Avermectins					
25 Substituent (R^2)	Sub-class	m.p. °C.	Theoretical Mol. Wt.	$(M + Na)^+$ From FAB Mass Spec.	m/e for Principle Fragments in the EI Mass. Spec.
					127, 113, 95 and 87.
	B1	145–150	882	905	576, 315, 261, 257, 231, 203, 179, 145, 127, 113, 95 and 87.
1-Methylbutyl	A1	—	900	923	594, 470, 319, 275, 257, 207, 183, 145, 127, 113, 95 and 87.
	B1	148–150	886	909	580, 337, 319, 261, 257, 253, 225, 207, 183, 145, 127, 113, 111, 95 and 87.
2-Thienyl	B1	152–154	898	921	592, 331, 257, 247, 219, 195, 145, 127, 113, 95 and 87.
4-Tetrahydropyranyl	A1	175–176	914	937	608, 333, 275, 249, 221, 197, 145, 127, 113, 95, and 87.
	A2	220 (dec)	932	955	351, 333, 275, 267, 249, 239, 221, 197, 145, 127, 113, 95 and 87.
	B1	177–183	900	923	594, 333, 249, 197, 145, 127, 113, 95 and 87.
	B2	173–178	918	941	612, 351, 333, 267, 261, 249, 239, 221, 207, 197, 145, 127, 113, 95 and 87.
	H_2B1*	160–163	902	925	486, 335, 269, 261, 257, 251, 223, 199, 145, 127, 113, 95 and 87.
1-Methyl-3-methoxypropyl	B1	143–150	902	925	596, 335, 257, 251, 223, 199, 145, 127, 113, 95 and 87.
1-Methylbut-3-ynyl	B1	95–100	882	905	576, 466, 315, 261, 257, 231, 203, 179, 145, 127, 113, 95 and 87.
	B2	107–110	900	923	594, 466, 333, 315, 261, 257, 249, 231, 221, 203, 179, 145, 127, 113, 95 and 87.
3-Cyclopentenyl	B1	150–152	882	905	576, 315, 261, 257, 248, 239, 231, 211, 203, 179, 145, 127, 113, 95 and 87.
3,4-Dihydro-pyran-2-yl	A1	130–135	912	935	331, 275, 257, 247, 219, 195, 145, 127, 113, 95 and 87.

*H_2B1 = dihydro B1 derivative. Prepared from corresponding B1 derivative by the procedure of Example 20.

EXAMPLE 19

25-Cyclobutyl-22,23-dihydro-avermectin B1

The product of Example 11 in benzene is hydrogenated in the presence of tris(triphenylphosphine)rhodium (I) chloride according to the procedure of EP-A-0001689 to yield the corresponding compound of formula (I) wherein each of R and R^1 is H. The product of Example 12 is similarly converted to the corresponding dihydro derivative.

EXAMPLE 20

25-Cyclohexyl-22,23-dihydro-avermectin B1

Dry benzene (200 ml) was purged first with a stream of nitrogen, then hydrogen. Tris(triphenylphosphine)rhodium (I) chloride (Wilkinson's catalyst) (665 mg) was then added. The passage of hydrogen was continued until the solution was yellow, and then for a further 10 minutes. 25-Cyclohexyl-avermectin B1 (2.010 g) was then added under a nitrogen blanket, and hydrogen bubbled through the solution for 24 hours. The solution was then evaporated to dryness. The residue was dissolved in methanol (50 ml) and evaporated; this was repeated. The residue was extracted with two portions of a 3:1 ether:hexane mixture (2×100 ml), and filtered. The combined filtrates were evaporated to dryness and chromatographed over silica gel (250 g of 230–900 mesh), eluting with an ether:methanol mixture (9:1). The relevant fractions were combined and evaporated to dryness to give crude product (2.25 g). This was purified using preparative HPLC, in three batches of 750 mg each, on a 42 mm×30 cm Dynamax column, eluting initially with methanol:water (85:15), graduating to methanol:water (83:17) over 15 minutes, at a flow rate of 95 ml/min. Appropriate fractions were pooled and evaporated to give the title compound (1.43 g; 81%) as a white powder, m.p. 150° C. (dec.). (See Table 1 for additional characterizing data.)

EXAMPLE 21

13-Deoxy-25-cyclopentyl-avermectin A2-aglycone

The product of Example 1 is treated with dilute sulphuric acid at room temperature and the resulting aglycone product is isolated and reacted with t-butyldimethylsilylchloride in dimethylformamide to provide the 23-O-t-butyldimethylsilyl aglycone derivative. This is dissolved in methylene chloride containing 4-dimethylaminopyridine and diisopropylethylamine, cooled in ice and treated dropwise with 4-nitrobenzenesulphonylchloride to yield the 13-chloro-13-deoxy product. This is finally dehalogenated by reaction with tributyltinhydride and deprotected with methanol contain-

ing a trace of paratoluene sulphonic acid following the procedures described in EP-A-0002615 to provide the compound of the formula I wherein each of R, R^1 and R^4 is H, R^3 is OH, and R^2 is cyclopentyl. In like manner, the compounds of Examples 7–10 and 13–20 are converted to the corresponding 13-deoxy derivatives.

EXAMPLE 22

Drench Formulation

The product of any one of the preceding Examples was dissolved in polyethylene glycol (average molecular weight 300) to give a solution containing 400 micrograms/ml for use as a drench formulation.

EXAMPLE 23

Anthelmintic Activity

Anthelmintic activity was evaluated against *Caenorhabditis elegans* using the in vitro screening test described by K. G. Simpkin and G. L. Coles in Parisitoloqy, 1979, 79, 19. The products of Examples 1, 7 and 9–16 all killed 100% of the worms at a well concentration of 0.1 micrograms per ml.

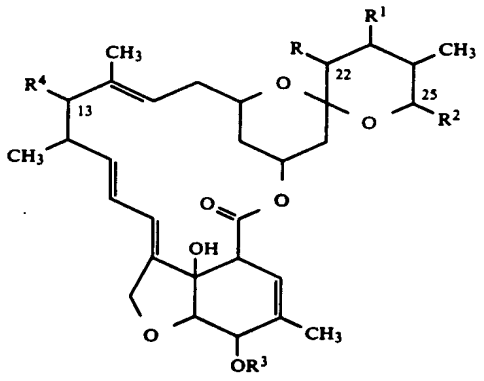
EXAMPLE 24

Insecticidal Activity

Activity against adult house fly *Musca domestica* is demonstrated using a standard test procedure in which flies are anaesthetised under carbon dioxide and 0.1 microliters of acetone containing the test compound is deposited on the thorax of female flies. The product of Examples 1, 7 and 9–16 all killed 100% of the treated flies at a dose of 0.01 micrograms per fly.

We claim:

1. A compound having the formula



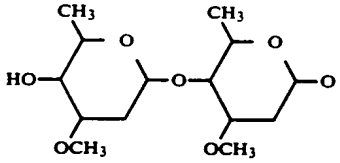
(I)

wherein R when taken individually is H; R^1 when taken individually is H or OH; R and R^1 when taken together represent a double bond;

R^2 is an alpha-branched C_4–C_8 alkynyl, C_3–C_8 alkoxyalkyl or C_3–C_8 alkylthio group; a C_5–C_8 cycloalkylalkyl group wherein the alkyl group is an alpha-branched C_2–C_5 alkyl group; a C_3–C_8 cycloalkyl or C_5–C_8 cycloalkenyl group, either of which may be substituted by methylene or one or more C_1–C_4 alkyl groups or halo atoms; or a 3 to 6 membered oxygen or sulphur containing heterocyclic ring which may be saturated, or fully or partially unsaturated and which may be substituted by one or more C_1–C_4 alkyl groups or halo atoms;

R^3 is hydrogen or methyl;

R^4 is H or a 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy group of the formula:



2. A compound according to claim 1 wherein R^4 is 4'-(alpha-L-oleandrosyl)-alpha-L-oleandrosyloxy.

3. A compound according to claim 2 wherein R is H and R^1 is H or OH.

4. A compound according to claim 3 wherein R^2 is a C_3–C_8 cycloalkyl which may be substituted by a C_{1-4} alkyl or a halo group.

5. The compound according to claim 4 wherein R is H; R^1 is OH; R^3 is methyl and R^2 is cyclopentyl.

6. The compound according to claim 4 wherein R is H; R^1 is OH; R^3 is methyl and R^2 is cyclohexyl.

7. The compound according to claim 4 wherein R is H; R^1 is OH; R^3 is methyl and R^2 is cyclobutyl.

8. The compound according to claim 4 wherein R is H; R^1 is OH; R^3 is H and R^2 is cyclobutyl.

9. The compound according to claim 4 wherein R is H; R^1 is OH; R^3 is methyl and R^2 is 2-methylcyclopropyl.

10. A compound according to claim 3 wherein R^2 is C_{5-8} cycloalkenyl.

11. The compound according to claim 10 wherein R is H; R^1 is OH; R^3 is methyl and R^2 is cyclohex-3-enyl.

12. A compound according to claim 3 wherein R^2 is a 3 to 6 membered oxygen or sulfur containing heterocyclic ring which may be saturated or unsaturated or substituted by a halo group.

13. The compound according to claim 12 wherein R is H; R^1 is OH; R^3 is methyl and R^2 is 3-thienyl.

14. The compound according to claim 12 wherein R is H; R^1 is OH; R^3 is methyl and R^2 is 2-furyl.

15. A compound according to claim 3 wherein R^2 is alkylthioalkyl.

16. The compound according to claim 15 wherein R^2 is 1-methylthioethyl; R^1 is OH and each of R and R^3 is hydrogen.

17. A compound according to claim 2 wherein R and R^1 taken together represent a double bond.

18. A compound according to claim 17 wherein R^2 is a C_3–C_8 cycloalkyl group.

19. The compound according to claim 18 wherein R^2 is cyclohexyl and R^3 is hydrogen.

20. The compound according to claim 18 wherein R^2 is cyclopentyl and R^3 is hydrogen.

21. The compound according to claim 18 wherein R^2 is cyclobutyl and R^3 is hydrogen.

22. A compound according to claim 17 wherein R^2 is a 3 to 6 membered oxygen or sulfur containing heterocyclic ring which may be saturated or unsaturated.

23. The compound according to claim 22 wherein R^2 is 3-thienyl and R^3 is methyl.

24. The compound according to claim 22 wherein R^2 is 3-thienyl and R^3 is hydrogen.

25. The compound according to claim 22 wherein R^2 is 3-furyl and R^3 is hydrogen.

26. A compound according to claim 17 wherein R^2 is a C_5–C_8 cycloalkenyl group.

27. The compound according to claim 26 wherein R^2 is cyclohex-3-enyl and R^3 is hydrogen.

28. A compound according to claim 2 wherein each of R and R^1 is H.

29. A compound according to claim 28 wherein R^2 is a C_3–C_8 cycloalkyl group.

30. The compound according to claim 29 wherein R^2 is cyclohexyl and R^3 is H.

31. The compound according to claim 29 wherein R^2 is cyclopentyl and R^3 is H.

32. The compound according to claim 29 wherein R^2 is cyclobutyl and R^3 is H.

33. A composition for the treatment and prevention of parasitic infections in humans and animals which comprises an antiparasitically effective amount of a compound of claim 1 together with an inert diluent or carrier.

34. A composition according to claim 33 in the form of a liquid drench or an oral or injectable formulation.

35. A composition according to claim 33 in the form of an animal feedstuff or a premix or supplement for addition to animal feed.

36. A method of combatting parasite infections or infestations which comprises contacting the organism responsible for said infection or infestation or the location of said organism with an antiparasitic amount of a compound according to claim 1.

* * * * *

EXHIBIT B

UNITED STATES PATENT AND TRADEMARK OFFICE

CERTIFICATE OF CORRECTION

PATENT NO. : 5,089,480
DATED : February 18, 1992
INVENTOR(S) : Stephen P. Gibson et al.

It is certified that error appears in the above-indentified patent and that said Letters Patent is hereby corrected as shown below:

On the title page item [56], "U.S. Patent No. 4,423,204" should read -- 4,423,209 --.

Signed and Sealed this

Twenty-third Day of January, 1996

Attest:

Attesting Officer

BRUCE LEHMAN

Commissioner of Patents and Trademarks

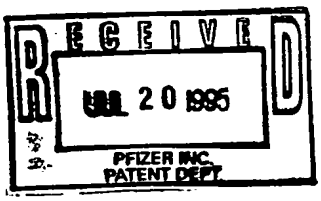
EXHIBIT C

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D. C. 20231

PAYOR NUMBER
000127

75N4/0705

PFIZER INC.
PATENT DEPARTMENT
235 EAST 42ND STREET, FLOOR 20
NEW YORK, NY 10017-5755



MAINTENANCE FEE STATEMENT

The data shown below is from the records of the Patent and Trademark Office. If the maintenance fees and any necessary surcharges have been timely paid for the patents listed below, the notation "PAID" will appear in column 10, "status" below.

If a maintenance fee payment is defective, the reason is indicated by code in column 10, "status" below. An explanation of the codes appears on the reverse of the Maintenance Fee Statement. TIMELY CORRECTION IS REQUIRED IN ORDER TO AVOID EXPIRATION OF THE PATENT. NOTE 37 CFR 1.377. THE PAYMENT(S) WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION. IF PAYMENT OR CORRECTION IS SUBMITTED DURING THE GRACE PERIOD, A SURCHARGE IS ALSO REQUIRED. NOTE 37 CFR 1.20(k) and (l).

If the statement of small entity status is defective the reason is indicated below in column 10 for the related patent number. THE STATEMENT OF SMALL ENTITY STATUS WILL BE ENTERED UPON RECEIPT OF ACCEPTABLE CORRECTION.

ITM NBR	PATENT NUMBER	FEE CDE	FEE AMOUNT	SUR CHARGE	SERIAL NUMBER	PATENT DATE	FILE DATE	PAY YR	SML ENT	STAT
1	5,089,480	183	960	----	07/142,888	02/18/92	01/11/88	04	NO	PAID

If the "status" column for a patent number listed above does not indicate "PAID" a code or an asterisk (*) will appear in the "status" column. Where an asterisk (*) appears, the codes are set out below by the related item number. An explanation of the codes indicated in the "status" column and as set out below by the related item number appears on the reverse of the maintenance fee statement.

ITM NBR	ATTY DKT NUMBER
1	SPC6947/6970

DIRECT THE RESPONSE TOGETHER WITH ANY QUESTIONS ABOUT THIS NOTICE TO:
COMMISSIONER OF PATENTS AND TRADEMARKS, BOX M. FEE, WASHINGTON, DC 20231

PTOL-439 (REV. 4-88)

EXHIBIT D

AH0542

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administratic
Rockville MD 20857

RECEIVED
DEC 12

INAD 6317

Roderick B. Dougherty, D.V.M.
Manager, Regulatory Affairs
Animal Health Development
Pfizer
Central Research
Pfizer, Inc.
Eastern Point Road
Groton, Connecticut 06340

DEC -7 1988

Dear Dr. Dougherty:

We refer to your investigational new animal drug exemption dated October 21, 1988, for avermectin B_{1a} analog designated as an injectable broad spectrum parasiticide for use in cattle. The submission provides an environmental testing plan.

Thank you for preparing and submitting the environmental testing plan for your new avermectin B_{1a} analog, UK-67,994, for use in cattle. Given the class of compounds we agree that it is prudent to plan early the types of environmental testing to be undertaken.

We believe that your testing plan, predicated upon your product behaving similarly to ivermectin, is appropriate. There appear to be, however, a couple of basic physical-chemical parameters that have been overlooked and there have also been some new developments in the area of the environmental toxicology of ivermectin that should also be considered for your compound.

We did not see mention of any plans to conduct a quantitative water solubility or dissociation constant determination with your compound. These are important prerequisite tests to complete prior to conducting most of the tests in your testing plan. Technical assistance for both tests is included in the FDA Environmental Assessment Technical Handbook.

A possibly important environmental toxicology problem has been raised with ivermectin since its approval for use in cattle. As you may know, dung-breeding beetles and flies fill important roles in the degradation and recycling of cattle wastes, particularly in arid pastureland. Dung from

calves treated with ivermectin failed to degrade in the expected time (100 days) in one study due to toxicity to dung beetles and dung breeding flies (Richard Wall and Les Strong, 1987. Environmental consequences of treating cattle with the antiparasitic drug ivermectin. Nature. 327:418-421 (4 June)). You should consider this possible effect of using your product in pasture cattle, and if such use is planned, consider conducting toxicity tests with dung beetles and/or dung breeding flies.

Sincerely yours,

Donald A. Gable

Donald A. Gable, D.V.M.
Director, Division of Therapeutic
Drugs for Food Animals
New Animal Drug Evaluation
Center for Veterinary Medicine

EXHIBIT E

BRIEF DESCRIPTION OF REPRESENTATIVE
SIGNIFICANT ACTIVITIES DURING THE
REGULATORY REVIEW PERIOD FOR DORAMECTIN

DATE	ACTIVITY	COMMENTS*
10/21/88	Submission to CVM	Request for INAD Number. Request for Review of EA plan.
10/26/88	Letter from CVM	Acknowledgment of 10/21/88 submission. Assignment of INAD No. 6317.
12/07/88	Letter from CVM	CVM sent comments on environmental test plan.
08/03/89	Submission to CVM	Submission of toxicity data. Request for permission to treat 5,000 cattle.
10/12/89	Submissions to CVM	NODS . Up to 4 x 25 ml and up to 2 x 25 ml vials to be used as antiparasitic in cattle.
10/24/89	Submission to CVM	Protocol amendment. Protocol for conduct of a photodegradation in water study.
10/25/89	Submission to CVM	NODS. Trial No. 1032C-60-89-004.
11/07/89	Submission to CVM	Applicant sought comments on studies of photodegradation, effect on seed germination and effect on seed growth.
11/20/89 11/21/89 12/12/89 01/30/90	Submissions to CVM	NODS. Trials Nos. 1032C-60-89-009. 1032C-60-89-011, 1032C-02-89-007 and 1032C-60-89-008.
02/01/90 02/06/90	Letters from CVM	Referred to review of protocols and environmental test plan
02/28/90 03/15/90 03/20/90 03/21/90 03/27/90	Submissions to CVM	NODS. Trials Nos. 1032C-60-90-015, 1032C-60-90-016, 1032C-60-90-014, 1032C-60-90-019, 1032C-60-90-013, 1032C-60-90-021, 1032C-60-90-018 and 1032C-60-90-024.
03/27/90	Letter from CVM	Provided comments and recommendations on the protocol; changes are necessary.
03/27/90	Submission to CVM	Commenting on and agreeing to the proposed changes to the protocol.
03/28/90 04/12/90	Submissions to CVM	NODS. Trials Nos. 1032C-60-90-023 and 1032C-60-90-022.
04/26/90	Submission to CVM	Applicant suggested to CVM that it would be beneficial to meet with CVM to discuss environmental safety testing program for doramectin.

* For explanation of abbreviations, see last page.

DATE	ACTIVITY	COMMENTS*
05/02/90 06/11/90	Submissions to CVM	NODS. 1% injectable solutions.
06/11/90	Letter from CVM	Response to applicant's submission of 04/26/90; referring to meeting held 05/0790.
06/13/90	Submission to CVM	Re environmental safety; concerned meeting of 05/07/90 regarding influence of doramectin on cow pat degradation.
06/22/90 06/29/90 07/31/90 08/16/90	Submissions to CVM	NODS. Trials Nos. 1031C-60-90-003, 1430C-60-90-003, 1430C-60-90-007 and 1233C-60-90-009.
08/29/90	Submission to CVM	Referring to clinical field trial program for ectoparasites; query as to what additional work is needed.
09/04/90 09/11/90 09/12/90	Submissions to CVM	NODS. Trials Nos. 1233C-60-90-001, 1233C-60-90-003 and 1232C-60-90-010.
09/19/90	Letter from CVM	Referred to submission of 06/13/90. Discussed design of a protocol for studying influence of doramectin treatment on cow pat degradation.
09/20/90 10/25/90 10/29/90 11/13/90	Submissions to CVM	NODS. Trials Nos. 1233C-60-90-008, 1232C-60-90-011, 1233C-60-90-007 and 1032C-60-90-025.
11/19/90	Submission to CVM	Doramectin 1% injectable antiparasitic for cattle withdrawal period reduction (2 volumes).
11/27/90 12/14/90	Submissions to CVM	NODS. Trials Nos. 1032C-60-90-029 and 1032C-60-90-030.
01/03/90	Submission to CVM	Doramectin 1% injectable antiparasitic for cattle amended authorization request; asking for expeditious review .
01/23/91 01/28/91 02/07/91 02/11/91	Submissions to CVM	NODS. Trials Nos. 1033C-60-91-001, 1033C-60-91-002, 1033C-60-91-004, 1032C60-90-032 and 1032C-60-90-031.
02/11/91	Submission to CVM	Segment 1 reproductive safety study with doramectin in cattle submitted. Meeting to review details with CVM requested.
02/26/91 03/01/91 03/04/91 03/05/91 03/11/91	Submisions to CVM	NODS. Trials Nos. 1032C-60-90-033, 0132C-60-90-028, 1033C-60-91-003 (and others).
03/13/91	Submission to CVM	Submission of minutes of meeting of 03/01/91 re environmental safely issues raised by CVM on 06/11/90 and 09/19/90

DATE	ACTIVITY	COMMENTS*
03/19/91	Submission to CVM	NODS. Shipment of 24 x 25 ml vials of 1% doramectin injectable solution.
04/26/91	Submission to CVM	Environmental safety submission
05/22/91	Submissions to CVM	NODS. Trial No. 1436N-60-91-008.
07/30/91	Submission to CVM	Laboratory animal and genetic toxicology studies submitted to CVM. This completed the requirements for the NADA.
08/06/91	Submission to CVM	NODS. Trial No. 0131C-60-90-004.
09/12/91	Submission to CVM	Phased environmental safety submissions. Submission of three final reports and summaries thereof for inclusion in EA.
10/04/91 10/11/91 12/05/91 01/15/92	Submissions to CVM	NODS. Trials Nos. 1532N-60-91-007, 2239A-60-91-055, 2039A-69-91-053, 2230A-60-91-054, 1031C-60-90-005 and 1032C-69-92-035.
03/05/92	Submission to CVM	Protocol for reproductive safety study (organogensis and pregnancy).
04/03/92 04/09/92	Submission to CVM	NODS. Trials Nos. 1436N-69-91-009 and 1434N-60-92-003.
05/05/92	Submission to CVM	Applicant responded to CVM comments on environmental safety studies and supplemented the original study.
06/04/92	Submissions to CVM	NODS. Trial No. 2039A-60-92-021.
07/27/92	Letter from CVM	CVM response to residue depletion study; confirmed that radio tracer is metabolically stable.
09/17/92 10/01/92	Submissions to CVM	NODS. Trials Nos. 2239A-60-92-027 and 2039A-60-92-026.
10/13/92 10/14/92	Letters from CVM	CVM response on animal toxicology.
12/01/92	Letter from CVM	CVM response to phased environmental submissions dated 05/08/92 and 06/22/92.
02/10/93	Submission to CVM	Protocol re reproductive study in bulls sent to CVM for review.
04/12/93	Letter from CVM	Comments on reproductive safety protocol; 1% injectable solution in bulls.
05/24/93	Submission to CVM	Supplying raw data on diskettes in response to CVM request.
06/04/93	Submissions to CVM	Manufacturing chemistry component and arithmetic means from nematode efficacy study submitted for CVM review.
06/28/93 07/02/93 07/28/93	Submissions to CVM	NODS. Trials Nos. 2539A-60-93-006, 1436N-60-92-012 and 2839A-60-93-003.
08/27/93	Submission to CVM	Environmental studies submission amendment.

DATE	ACTIVITY	COMMENTS*
09/24/93	Telecons	Discussed requirements for analytical method for doramectin in milk and pivotal efficacy studies for FOI summary.
10/01/93	Submission to CVM	NODS. Trials Nos. 2239A-60-92-027 and 1535N-60-93-016.
12/22/93	Telecon	Re: foreign data; arithmetic means; and efficacy protocols.
01/26/94	Submission to CVM	Minutes of 01/19/94 EA meeting.
02/28/94 03/08/94 03/10/94 03/15/94 03/17/94	Telecons	Re: residue metabolism review status; labeling restrictions; milk residue data.
03/31/94	Submission to CVM	Reproductive safety study-final report.
04/07/94 04/18/94 04/25/94	Submission to CVM	NODS. Trials Nos. 1533N-60-94-002, 2239A-60-94-003, 2239A-60-94-007 and 1436N-60-92-012.
06/20/94	Letter from CVM	Environmental studies response. Refers to submission dated 08/06/93.
07/20/94	Submission to CVM	Submission of full reports of four toxicity studies in response to request from CVM.
08/29/94	Submission to CVM	Memorandum of Conference. Refers to 08/22/94 meeting re human food safety issues
09/13/94 09/16/94	Submissions to CVM	NODS. Trials Nos. 2829A-60-94-051 and 2839A-60-94-050.
10/17/94	Letter from CVM	CVM review of drug metabolism meeting. Refers to submission of 08/29/94
11/30/94 12/01/94 12/12/94 01/05/95	Submissions to CVM	NODS. Trials Nos. 2239A-60-94-068, 2239A-60-94-067, 1532N-60-94-160 and 2039A-60-95-002.
01/06/95	Submission to CVM	Revised determinative assay procedure (quantification of doramectin in cattle liver).
01/10/95 01/18/95	Submission to CVM	NODS. Trials Nos. 2239A-60-94-070 and 2239A-60-94-038.
01/30/95	Submission to CVM	Request review of nematode persistent efficacy protocol.
03/01/95	Telecon	Re: toxicology FOI summary.
03/02/95 03/17/95 03/29/95 04/05/95	Submissions to CVM	NODS. Trials Nos. 2239A-60-95-010, 1231C-60-95-190, 1231C-60-95-191, 2239A-60-95-022 2239A-60-95-026 and 2239A-60-95-027.
04/06/95 04/07/95	Submissions to CVM	Requesting review of FOI summaries relating to efficacy and toxicology.
04/25/95	Submission to CVM	NODS. Trial No. 2239A-60-95-028.

DATE	ACTIVITY	COMMENTS*
04/28/95	Submission to CVM	Requesting a revised doramectin determinative analytical procedure.
05/04/95	Submission to CVM	NODS. Trial No. 2039A-60-96-016.
06/08/95	Submission to CVM	Animal disposition notice. Study No. 1532N-60-94-160.
07/06/95	Submission to CVM	Requested review of two persistent efficacy studies which support persistent efficacy claim.
07/18/95	Letter from CVM	CVM assignment of liver tolerance and withdrawal time (submission dated 10/28/94)
08/10/95	Submission to CVM	NODS. Trial No. 1231C-60-95-198.
08/14/95	Letter from CVM	Review of full reproductive safety study (bulls).
08/15/95 09/05/95 09/07/95 09/11/95	Submission to CVM	NODS. Trials Nos. 1231C-60-95-285, 2239A-60-95-049, 2239A-60-95-048 2239A-60-95-048 and 2239A-60-95-049.
09/22/95	Telecon	Applicant asked CVM for guidance on NADA format.
10/02/95 10/10/95	Submissions to CVM	NODS. Trials Nos. 1231C-60-95-196 and 1430C-60-95-213.
10/19/95	Submissions to CVM	Animal Disposition Notices
10/31/95	Submission to CVM	Bull Reproductive Safety - Memorandum of Conference.
11/21/95 11/30/95 12/06/95	Submissions to CVM	NODS. Trials Nos. 2239A-60-95-156, 2039A-60-95-041 and 2039A-60-95-002.
12/21/95	Submission to CVM	Requested meeting with Chemotherapeutics Branch Chief re chemistry and manufacturing controls issues raised by CVM.
01/17/96	Submission to CVM	NODS. Trial No. 2039A-60-95-058.
01/29/95 01/31/96	Telecons	Re: analytical methods trial and CMC issues
02/01/96	Letter from CVM	Acceptance of dairy heifer label statement
02/13/96	Submission to CVM	Animal Disposition Notice
03/04/96	Telecon	CVM was informed that NADA was about to be filed and ask if complete for feasible Chemistry FOI Summary
03/07/96	Submission to CVM	NADA filed.
03/12/96	Letter from CVM	Acknowledgment of NADA Submission and assignment of NADA No. 141-061.
03/27/96	Telecon	CVM was contacted to indicate that swine label was being finalized for inclusion in boar target animal safety submission.
04/05/96	Telecon	Dr. Vaughn stated that CVM was re-evaluating the efficacy section reviews, and that FOI may need to be revised.

DATE	ACTIVITY	COMMENTS*
04/24/96	Telecon	Re: Label review status and FOI format.
04/25/96	Submission to CVM	Animal Disposition Notice.
05/14/96	Letter from CVM	Regulatory method approval.
05/31/96	Telecon	CVM was informed that applicant had completed filter challenge work and report would be filed soon.
06/03/96	Telecons	Re: human food safety, human food safety FOI summary.
06/04/96	Submissions to CVM	Animal Disposition Notice.
06/11/96 06/19/96 06/21/96 06/28/96	Telecons	Several labeling issues discussed.
07/01/96	Telecon	Pre-approval inspection.
07/09/96	Telecon	CVM given information about when final labelling would be submitted.
07/15/96	Telecon	Dr. Benson was contacted for update on NADA approval package.
07/30/96	Letter from CVM	Approval of NADA No. 141-061.

ABBREVIATIONS

INAD	Investigational New Animal Drug
NADA	New Animal Drug Application
CVM	Center for Veterinary Medicine
EA	Environmental Assessment
NODS	Notice of Drug Shipment
CMC	Chemistry and Manufacturing Controls
FOI	Freedom of Information

PATENT
PC6947AJTL

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE:	U.S. PATENT NO.: 5,089,480 :
ISSUED:	FEBRUARY 18, 1992 :
TO:	STEPHEN P. GIBSON ET AL. :
FOR:	ANTIPARASITIC AGENTS :
FROM:	SERIAL NO. 142,888 :
OF:	JANUARY 11, 1988 :

Hon. Commissioner of Patents and Trademarks
Box Patent Extension
Washington, DC 20231

Sir:

DECLARATION ACCOMPANYING APPLICATION FOR
EXTENSION OF PATENT TERM UNDER 35 U.S.C. §156

I, J. TREVOR LUMB, declare as follows.

1. I am a patent attorney. I am a member of the Bar of the State of New York and I am authorized to practice before the Patent and Trademark Office, Registration No. 28,567.

2. I am employed by PFIZER INC., a corporation of Delaware, having a place of business at 235 East 42nd Street, New York, NY 10017. PFIZER INC. is the owner of record of United States Patent No. 5,089,480.

3. I have general authority from PFIZER INC. to act on its behalf in patent matters.

4. I have reviewed and I understand the contents of the application of PFIZER INC., dated September 25, 1996, which is being submitted herewith for extension of the term of United States Patent No. 5,089,980 under 35 U.S.C. §156 and 37 C.F.R. §1.730.

5. I believe that United States Patent No. 5,089,480 is subject to extension pursuant to 37 C.F.R. §1.710.

6. I believe that the length of extension of term of United States Patent No. 5,089,480 which is being claimed by PFIZER INC. is justified under 35 U.S.C. §156 and the applicable regulations.

7. I believe that the patent for which extension is being sought meets the conditions for extension of the term of the patent as set forth in 37 C.F.R. §1.720.

I declare further that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 oF Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application being submitted herewith or any extension of patent term granted thereon.

Signed this 25th day of September, 1996, at New York, New York.

J. Trevor Lumb

J. Trevor Lumb
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